Association Analysis between Five Microsatellite Loci and Litter Size in Small Tail Han Sheep*

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ABSTRACT : The objective of the present study was to explore associations between five microsatellites linked to Fec^B and $FecX^I$ genes and litter size in Small Tail Han sheep. The polymorphisms of five microsatellite loci, OarAE101, BM1329, BMS2508, TGLA54 and TGLA68 were detected in 244 ewes of Small Tail Han sheep. Analysis of association between three microsatellite loci (BMS2508, BM1329 and OarAE101) located in the 10 cM region covering the Fec^B gene (*Booroola* gene) and litter size in Small Tail Han sheep indicated that BMS2508 had significant effect on litter size in the second parity ($p<0.05$), but no significant effect on litter size in the first parity ($p>0.05$), while the other two microsatellite loci had no significant effect on litter size in both the first and the second parity in Small Tail Han sheep ($p>0.05$). At microsatellite locus BMS2508, least squares means in the second parity of genotypes 101/111 and 99/109 were significantly higher than those of genotypes 99/99, 99/101, 99/111 and 99/115 ($p<0.05$); least squares mean in the second parity of genotype 101/111 was significantly higher than that of genotypes 109/111 and 111/111 ($p<0.05$). Results of this study also indicated that two microsatellite loci (TGLA54 and TGLA68) that confined the 28.7 cM region covering the $FecX^I$ gene (*Inverdale* gene) did not affect litter size in both the first and the second parity in Small Tail Han sheep significantly ($p>0.05$). The information found in the present study is very important for improving the reproductive performance in sheep breeds by marker assisted selection. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 11 : 1555-1559)***

Key Words : Sheep, Litter Size, Microsatellite, Polymorphism, Least Squares Analysis

INTRODUCTION

Because of the low heritability of litter size in sheep, selective breeding is relatively in effective. Marker assisted selection (MAS) could improve the efficacy of selective breeding for traits with low heritability by working on selection time, selection strength and accuracy. Finding molecular markers linked to quantitative trait loci is the first step of MAS. Microsatellite is an excellent molecular marker because of its large numbers and even distribution in the genome and high polymorphism.

The *Booroola* sheep was a highly prolific strain of Merino, initially developed in Australia by two commercial sheep breeders, the Seears brothers of '*Booroola*', New South Wales and later by the Divisions of Animal Genetics and Animal Production of the Commonwealth Scientific and Industrial Research Organization. Researches in New Zealand and Australia confirmed the single gene inheritance of high prolificacy in *Booroola* sheep. The locus was named as Fec^B (Fec=fecundity, B=*Booroola*) by the Committee on Genetic Nomenclature of Sheep and Goats. The effect of the Fec^B gene is additive to ovulation rate and partially dominant to litter size (Davis et al., 1982; Piper et al., 1985). One copy of the Fec^B gene increases ovulation rate by 1.3 to 1.6 and two copies by 2.7 to 3.0; litter size is increased by 0.9 to 1.2 in ewes carrying a single copy and 1.1 to 1.7 in ewes with two copies of Fec^B gene (Davis et al., 1982; Piper et al., 1985). Average ovulation rates of *Booroola* ewes of genotypes BB (Fec^B/Fec^B), B+ (Fec^B/Fec^+) and ++(Fec^+/Fec^+) were 4.7, 2.9 and 1.5, respectively (Piper et al., 1985). The Fec^B gene has been mapped to ovine chromosome 6 (Montgomery et al., 1993, 1994). Montgomery et al. (1993) found that microsatellite marker OarAE101 was linked to the Fec^B gene with a maximum lod (likelihood of the odds) score of 17.33 at a distance of 13 centimorgans (cM), microsatellite marker OarHH55 was linked to the Fec^B gene with a maximum lod score of 9.38 at a distance of 20 cM, the two microsatellite markers OarAE101 and OarHH55 were linked to each other with a maximum lod score of 30.40 at a distance of 5 cM. Lord et al. (1998) mapped the Fec^B gene to a 10 cM region between microsatellite markers BM1329 and OarAE101. Mulsant et al. (1998) reported that the closest flanking markers of the Fec^B gene were bovine microsatellite BMS2508 and caprine microsatellite LSCV043, which were situated about 2 cM on either side of the gene.

The *Inverdale* ($FecX^I$) gene was identified in a prolific family of Romney ewes in Invermay Agricultural Center in New Zealand in 1984. This family was progeny of one ewe A281 that had produced 33 lambs in 11 lambings (Davis et al., 1991). The *Inverdale* gene affects ovulation rate and ovarian development, and was located on the ovine X chromosome by family studies (Davis et al., 1991, 1992).

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Table 1. Primer sequences of five microsatellite loci

Locus	Source	Primer sequences (5'→3')
OarAE101	Montgomery et al., 1993	Forward: TTCTTATAGATGCACTCAAGCTAGG
		Reverse: TAAGAAATATATTTGAAAAAACTGTATCTCCC
BMS2508	G18959	Forward: TTTCTGGGATTACAAAATGCTC
		Reverse: TTTCTTAGGGGAGTGTTGATTC
BM1329	Bishop et al., 1994	Forward: TTGTTTAGGCAAGTCCAAAGTC
		Reverse: AACACCGCAGCTTCATCC
TGLA54	Galloway et al., 1996	Forward: CTCAATATTTTGCAATAACATATAAGG
		Reverse: ACGATATCATGTTAGTTTCAGGTG
TGLA68	Galloway et al., 1996	Forward: ATCTTACTTACCTTCTCAGAGCT
		Reverse: GGGACAAAATTTTACATATACACTT

Table 2. Characteristics of five microsatellite markers in Small Tail Han sheep

Locus	Number of alleles	Size range (bp)	Heterozygosity
BM1329	6	160 to 180	0.516
TGLA54	5	116 to 136	0.267
TGLA68	2	98 to 100	0.497
OarAE101	9	97 to 135	0.363
BMS2508	6	93 to 115	0.642

$FecX^I/FecX^I$ (II), $FecX^I/FecX^+$ (I+) and $FecX^+/FecX^+$ (++) are the three genotypes of ewes. Ovulation rates of I+ ewes are increased by about one egg per ewe lambing, and the resultant litter sizes are increased by 0.6 lambs per ewe lambing. Shackell et al. (1993) reported that the mean ovulation rate of I+ ewes (2.6±0.2) is significantly higher than that of ++ ewes (1.8±0.1) ($p<0.01$). McLeod et al. (1995) found that mean ovulation rates were significantly higher ($p<0.01$) in I+ ewes (3.1±0.3) than in ++ ewes (2.1±0.2). II ewes have small non-functional streak ovaries, and are infertile (Davis et al., 1992). The *Inverdale* gene was first located in 28.7 cM region flanked with microsatellites TGLA54 and TGLA68 and further mapped to a 10 cM region confined by three microsatellite loci INV07, INV12 and INV17 (Galloway et al., 1999). Recently the *Inverdale* gene was mapped to a 10 cM region flanked with microsatellite loci McM551 and OarMP1, TGLA54 is in this region and 1.4 cM away from OarMP1 (Galloway et al., 2000).

Small Tail Han sheep is a prolific sheep breed in P. R. China. The lambing percentage averaged 261% (Zheng, 1989) and 265.2% (Wang et al., 1990) in Small Tail Han sheep in Shandong Province, P. R. China. It was known that Fec^B and $FecX^I$ are two major genes controlling litter size of sheep. This study was conducted to examine whether these two genes are involved in the high prolificacy in Small Tail Han sheep by analyzing association between microsatellite loci linked to the two genes and litter size in Small Tail Han sheep.

MATERIALS AND METHODS

Genome DNA and microsatellite primers

Genome DNA used in this study was extracted from blood samples of 244 ewes of Small Tail Han sheep in Jiaxiang Breeding Sheep Farm in Shandong Province, P. R. China. Microsatellite primers were synthesized by referring to Bishop et al. (1994), Stone, Montgomery et al. (1993) and Galloway et al. (1996) (Table 1).

Microsatellite genotyping

The microsatellite genotyping was described in detail by Chu et al. (2002). The PCR reactions were performed in 10 μl volume reactions with 25 ng sheep genome DNA, 1.5 mM $MgCl_2$, 200 μM each dNTP, 0.2 μM each primers, 1×PCR buffer and 1.0 unit of Taq DNA polymerase. The PCR products were loaded to non-denatured polyacrylamide gel to electrophorese in 1×TBE buffer at 170 volt for 15 to 20 h. Gel was stained with silver nitrate (silver staining) after electrophoresis to read fragment sizes.

Statistical analysis

The following formula was used to compute heterozygosity.

$$h = 1 - \sum_{i=1}^{n} P_i^2$$

where, P_i is frequency of allele i in the locus, n is number of alleles in the locus.

The following statistical model was fitted to compare difference of litter size among microsatellite genotypes.

$$y_{ij}=\mu+ M_i + e_{ij}$$

where, y_{ij} is phenotypic value of litter size, μ is population mean, M_i is the fixed effect of the i^{th} genotype, and e_{ij} is random error effect of each observation. Calculations were performed using Proc GLM of SAS (Ver 8.1).

RESULTS

Characteristics of five microsatellite markers in Small Tail Han Sheep

The characteristics of five microsatellite markers in Small Tail Han sheep are summarized in Table 2. The

number of alleles ranged from 2 to 9 in Small Tail Han sheep; heterozygosity ranged from 0.267 to 0.642. The PCR amplified products of five microsatellites were detected by non-denatured polyacrylamide gel electrophoresis. The pictures for silver staining microsatellite genotyping were provided by Wang (2001).

Association between microsatellites and litter size in Small Tail Han sheep

Least squares means and standard errors of litter size for different genotypes of five microsatellite loci in Small Tail Han sheep are presented in Table 3.

From Table 3 it can be seen that: (a) microsatellite locus BMS2508 had a significant effect on litter size in the second parity ($p<0.05$), but no significant effect on litter size in the first parity ($p>0.05$). These results indicated that an interaction between BMS2508 genotypes and parities was present. LSM2 of BMS2508 genotypes 101/111 and 99/109 were significantly higher than those of genotypes 99/99, 99/101, 99/111 and 99/115 ($p<0.05$). LSM2 of

Table 3. Least squares mean (LSM) and standard error (SE) of litter size for different genotypes of five microsatellite loci in Small Tail Han sheep

Locus	Genotype***	No.of animals	LSM1*	SE1*	LSM2*	SE2*
BMS2508	93/111	5	2.20	0.35	2.60 ^{abc}	0.38
	99/99	59	2.27	0.10	2.53^{c}	0.11
	99/101	8	1.88	0.28	2.13 ^{c}	0.30
	99/109	10	2.00	0.25	3.10 ^{ab}	0.27
	99/111	79	2.16	0.09	2.33 ^{c}	0.10
	99/115	15	1.93	0.20	2.20 ^{c}	0.22
	101/111	10	2.60	0.25	3.20 ^{a}	0.27
	109/111	12	2.50	0.23	2.83 ^{bc}	0.25
	111/111	27	2.07	0.15	2.52 ^{bc}	0.16
	111/115	10	2.40	0.25	2.50 ^{abc}	0.27
BM1329	162/162	12	2.33	0.22	2.58	0.25
	162/164	99	2.16	0.08	2.44	0.09
	164/164	82	2.20	0.09	2.48	0.10
	164/166	12	2.17	0.22	2.58	0.25
	164/174	6	2.17	0.32	3.17	0.35
	164/180	6	2.33	0.32	3.00	0.35
OarAE101	97/97	154	2.17	0.06	2.51	0.07
	97/101	6	2.50	0.32	2.83	0.36
	97/107	14	2.21	0.21	2.43	0.23
	97/109	26	2.42	0.15	2.58	0.17
	97/115	6	1.50	0.32	2.50	0.36
	97/129	8	2.38	0.28	3.00	0.31
	97/135	11	2.27	0.24	2.18	0.26
	97/111	5	2.00	0.35	2.40	0.39
TGLA54	116/134	8	2.63	0.28	2.75	0.31
	132/134	14	2.28	0.21	2.57	0.23
	134/134	175	2.19	0.06	2.50	0.07
	134/136	33	2.21	0.14	2.36	0.15
	136/136	5	1.60	0.35	2.40	0.39
TGLA68	98/98	76	2.29	0.09	2.57	0.10
	98/100	112	2.13	0.07	2.45	0.08
	100/100	56	2.21	0.11	2.46	0.12
TGLA54/68**	116/134, 98/98	5	3.00	0.35	3.20	0.39
	132/134, 98/100	8	2.25	0.28	2.75	0.31
	134/134, 98/98	50	2.22	0.11	2.46	0.12
	134/134, 98/100	85	2.16	0.09	2.50	0.09
	134/134,100/100	40	2.23	0.12	2.58	0.14
	134/136, 98/98	12	2.25	0.23	2.75	0.25
	134/136, 98/100	14	2.00	0.21	2.07	0.23
	134/136,100/100	7	2.57	0.30	2.29	0.33

* LSM1 and SE1 indicate least squares mean and standard error of litter size in the first parity respectively; LSM2 and SE2 indicate least squares mean and standard error of litter size in the second parity respectively. Different letters of superscripts of least squares means of one microsatellite locus indicate significant difference between them ($p<0.05$). ** TGLA54 and TGLA68 are combined as one locus. *** This table only lists genotypes of more than five animals.

BMS2508 genotype 101/111 was significantly higher than that of genotypes 109/111 and 111/111 ($p<0.05$). Both LSM1 and LSM2 of genotype 101/111 were the highest among BMS2508 genotypes. Both LSM1 and LSM2 of BMS2508 genotype 99/101 were the lowest. (b) BM1329 had no significant effect on both LSM1 and LSM2 in Small Tail Han sheep ($p>0.05$). LSM2 of genotype 164/174 was the highest among BM1329 genotypes. Both LSM1 and LSM2 of genotype 162/164 were the lowest. (c) OarAE101 had no significant effect on both LSM1 and LSM2 in Small Tail Han sheep ($p>0.05$). LSM1 of genotype 97/101 was the highest among OarAE101 genotypes while LSM1 of genotype 97/115 was the lowest. LSM2 of genotype 97/129 was the highest among OarAE101 genotypes while LSM2 of genotype 97/135 was the lowest. (d) TGLA54 had no significant effect on both LSM1 and LSM2 in Small Tail Han sheep ($p>0.05$). Both LSM1 and LSM2 of genotype 116/134 were the highest among TGLA54 genotypes. (e) TGLA68 had no significant effect on both LSM1 and LSM2 in Small Tail Han sheep ($p>0.05$). Both LSM1 and LSM2 of genotype 98/98 were the highest among TGLA68 genotypes while both LSM1 and LSM2 of genotype 98/100 were the lowest. (f) Combining TGLA54 and TGLA68 as one locus, there were no significant differences for both LSM1 and LSM2 among different genotypes. Both LSM1 and LSM2 of genotype 116/134 and 98/98 were the highest among TGLA54/TGLA68 genotypes while both LSM1 and LSM2 of genotype 134/136 and 98/100 were the lowest. For BM1329, OarAE101, TGLA54 and TGLA68, interaction between genotypes and parities was absent.

DISCUSSION

We had no sequence information of microsatellite loci INV07, INV12 and INV17 that confined a 10 cM region covering the *Inverdale* gene (Galloway et al., 1999). We used microsatellite marker TGLA54 that is in the 10 cM region covering the *Inverdale* gene (Galloway et al., 2000) and TGLA68 that confined the *Inverdale* gene in a 28.7 cM region together with TGLA54 (Galloway et al., 1999).

Considering possible recombination between microsatellite markers confining a gene, it is better to combine the markers as one locus to conduct association analysis so that recombination between markers is detectable. In this study, TGLA54 and TGLA68 that confined the *Inverdale* gene in a 28.7 cM region were combined together and considered as one locus (TGLA54/68). Association analysis indicated no association between genotypes of TGLA54/68 locus and litter size in Small Tail Han sheep. For *Booroola* gene, the combinations of markers (BMS2508, BM1329 and OarAE101) linked to the gene are more than 100; many genotypes only had one animal. We did not combine markers linked to *Booroola* gene as one locus for association analysis.

BMS2508 is linked to the Fec^B gene at a distance of 2 cM (Mulsant et al., 1998). Our study indicated the association of BMS2508 and litter size of the second parity in Small Tail Han sheep ($p<0.05$). It is possible that the Fec^B gene is present in Small Tail Han sheep. Further evidence is needed to confirm this hypothesis, such as crossing Small Tail Han sheep with BB *Booroola* sheep. TGLA54 is in the 10 cM region that covers the *Inverdale* gene, and our study indicated TGLA54 had no association with litter size in Small Tail Han sheep ($p>0.05$). The *Inverdale* gene is probably not present in Small Tail Han sheep, and further evidence is also needed to confirm this.

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